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1993 - 1994	Research Fellow – University of Oxford, UK
1994 - 1997	PostDoc – Imperial Cancer Research Fund, London, UK
1997 - 2000	Senior Investigator, Bioinformatics, SmithKline Beecham R&D, Harlow, UK
2001 - 2009	Group Leader – European Molecular Biology Laboratory (EMBL), Heidelberg, Structural Biology Programme
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Rob Russell

Understanding and predicting mechanism of gene or protein variants

Goal

The advent of high-throughput sequencing and proteomics techniques means that thousands of variants or post-translational modifications can be obtained cheaply in a single experiment. While these data have provided new insights processes & diseases, they often fall short of providing a detailed understanding of disease progression or the underlying molecular mechanism that might enable the better design of novel diagnostics or therapeutics. Our group is currently developing and applying methods to understand and predict the impact of these gene or protein changes on protein function.

Background

Mutational events are central to all human genetic disorders and to all human cancers. The advent of high-throughput sequencing techniques (HTS) now means that thousands of mutations (e.g. from a tumour or a genetic disease sample) can be obtained cheaply in a single experiment. Similarly, the advent of high-throughput, sensitive proteomics methods now mean one can also sample the space of post-translational modifications (PTMs), such as phosphorylation or acetylation, just as rapidly. While these data have provided unprecedented insights into the nature of biological processes & diseases, they fall short of providing a detailed understanding of disease progression or the underlying molecular mechanism that might enable the better design of novel diagnostics or therapeutics. The increasing speed and decreasing cost of data generation by improvements in these technologies, moreover, means that the already wide gap between biological states for which data are available and those for which even the beginnings of a mechanistic understanding is known will continue to grow.

Rewiring is a frequently cited analogy for how mutations or PTMs affect biological systems. Proteins are arranged into pathways, complexes and (generally) networks, and mutations or PTMs often affect individual interactions, effectively rewiring the existing circuits. Disease mutations that specifically affect interactions have been termed edgetic to capture the notion that it is the edges (interactions) in a biological network that are affected above the individual nodes (e.g. proteins or genes). To study rewiring in more detail requires extensive knowledge about the molecular mechanism by which proteins interact with other molecules, which ultimately comes from three-dimensional structures. My group has spearheaded methods to rapidly predict protein-protein, protein-chemical, protein-peptide interactions by homology, and most recently to predict the effect of both mutations and PTMs on biomolecular interfaces, including protein-protein, protein-drug/small-molecule and protein-nucleic acids, and our newest approaches have been applied to a

number of cancer mutations and phosphorylation and acetylation datasets revealing, indeed, that it is possible, by a systematic synthesis of mutation/PTM data with interactome and 3D structures, to obtain information about precise rewiring events in diseases.

Research Highlights

Rewiring events mediated by phosphorylation or cancer mutations

To assess the impact of mutations or PTMs on biomolecular interfaces, we have devised a system to assess the Mechanistic Impact of Structural Modifications (mechismo.russellab.org) that scores changes in particular amino acids according to their likely effects on the local structural environment. An important aspect of this work is to survey large datasets to determine general attributes, such as the fraction of data impacting on protein-protein or protein-drug interfaces, or differences between datasets from different disease groups (e.g. different cancers; Figure 1).

We have also applied this approach systematically to the current set of Cancer mutations (COSMIC database) to identify a set of 213 biomolecular interfaces that are most often perturbed in cancers. These show distinct patterns in different cancer types, and some of them correlate with patient attributes, such as the presence of P53 zinc-binding site mutations, which are associated with poorer survival across all cancers considered (Figure 2).

Another main aim of the work is to prioritise mutations or PTMs as candidates for future studies. We have already tested numerous phosphorylation sites determined largely by high-throughput studies and identified as novel protein-protein interaction switch candidates using the two-hybrid system and are currently screening several

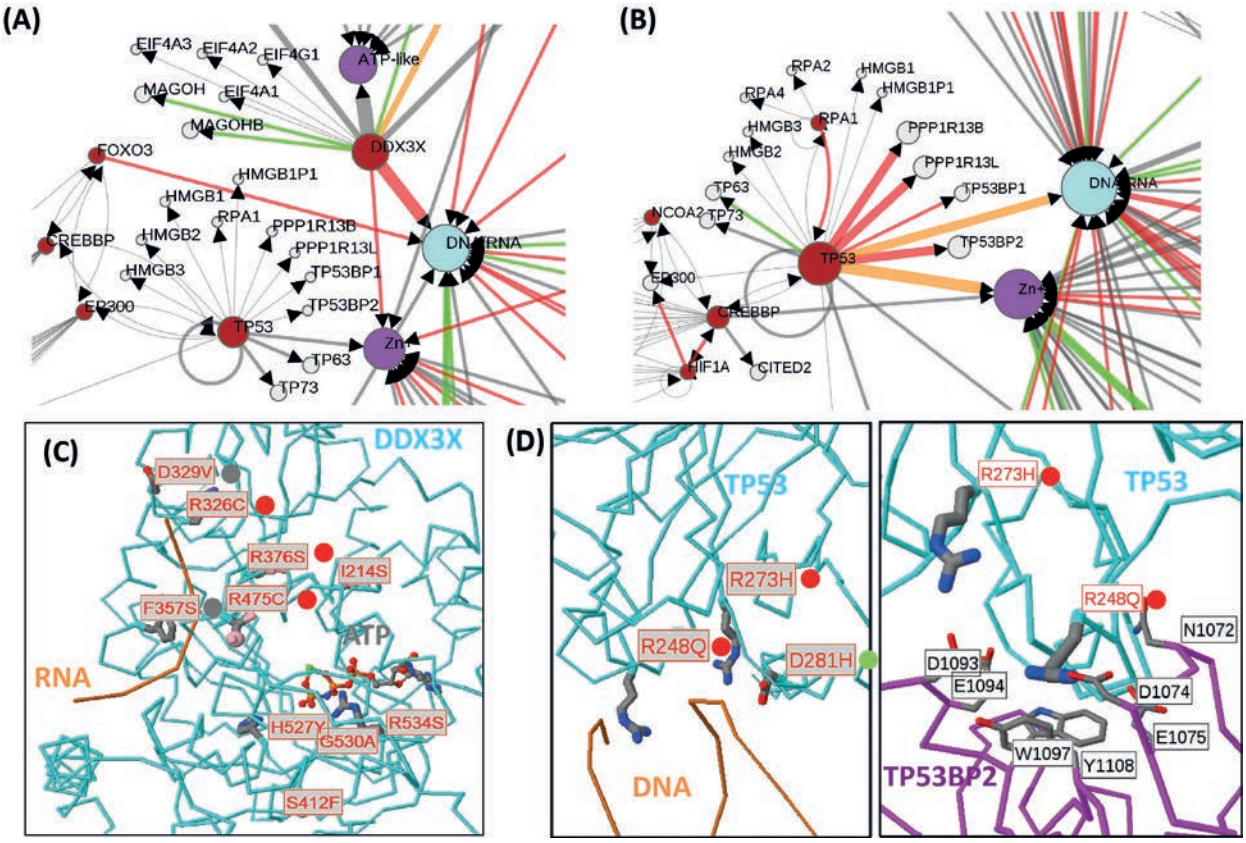

Figure 1: Variants in HTS cancer datasets
(A) *Portions of the wider network of interactions involving proteins (red if mutated, grey if not), chemicals (magenta) and DNA/RNA (blue) affected by mutations identified after sequencing Medulloblastoma tumors and Pancreatic cancer* ***(B).*** *The size of the red protein nodes is proportional to the number of variants contained within them, the size of chemical and DNA/RNA nodes is proportional to the number of sites predicted to interact with them, and the width of edges is proportional to the number of sites affecting them. Red edges are those where the effect of the mutations is predicted to diminish the interaction, green to enhance and orange where different mutations have opposite effects.* ***(C)*** *Structures of DDX3X showing Medulloblastoma mutations affecting DNA or ATP-binding, and* ***(D)*** *mutations in Pancreatic cancer affecting functional interactions of TP53 with DNA and TP53BP2.*

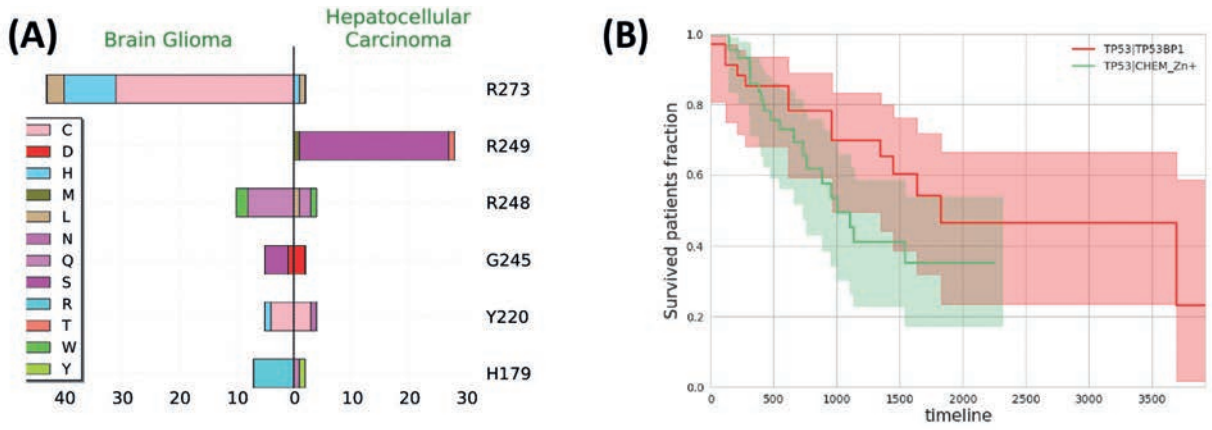

Figure 2: Interrogating mechanism within all cancer types

(A) *Differences in the position and nature of mutations within TP53 in Glioma and Hepatocellular carcinoma. The bars show the number of samples containing a particular motif, with the preferred amino acid substituents coloured as indicated. All of the residues shown (left of figure) are at either the DNA or regulatory protein interfaces.* ***(B)*** *Survival curve showing how tumors having mutation of the TP53 zinc binding site (red) have a poorer survival (across all cancers) compared to those having other TP53 mutations (green).*

dozen cancer mutations predict to have particular impacts on interactions with critical cancer signaling molecules such as GTPases and kinases.

Combining proteomics & genetics to interrogate a disease relevant organelle

Genetic diseases represent another where we use our tools to delineate mechanism and to relate it to disease variants & mutations. As part of an FP7 Systems Biology Project (Syscilia) in collaboration with 15 groups across Europe, combined affinity-proteomics/mass-spectrometry, genetics and cell biology to interrogate cilia: still poorly understood, cell signaling hubs whose defects cause ciliopathies, devastating heterogeneous genetic diseases.

We selected 217 known/candidate human ciliary proteins and using affinity tagging and mass-spectrometry we identified a high resolution landscape of 4905 interactions and 52 complexes involving 1319 proteins. The landscape provides unprecedented details of ciliary signalling and proteostasis, highlighting essential connections to ciliary and vesicle transport, the cytoskeleton, signaling, and ubiquitination (Figure 3).

We extended our Socioaffinity method originally developed for the Yeast proteome and because of the extensive reverse-tagging (i.e. where multiple components of a complex are tagged) and repeated experiments, it was able to delineate sub-complexes, including novel sub-complexes in Intraflagellar transport complex B (IFT-B), which we showed is indeed two complexes by sucrose density centrifugation and by EPASIS, which couples MS-proteomics to increasing concentrations of denaturants. Using the Mechismo approach (above) and binding site hot-spots we also identified several variants in very severe ciliopathies that specifically disrupted different sub-complexes as confirmed by subsequent comparisons between wt and variant proteins.

The interactome included many (over 1000) proteins not previously known to be ciliary overlaps and intriguingly several of these are known, when mutated, to cause genetic diseases not known to be ciliary, and for one (3M Syndrome) we showed that three known causative genes affect ciliogenesis (when knocked-down), and that fibroblasts from patients lack cilia.

Selected Publications

Raimondi F, Singh G, Betts MJ, Apic G, Vukotic R, Andreone P, Stein L, Russell RB. Insights into cancer severity from biomolecular interaction mechanisms. Sci Rep. 2016 Oct 4;6:34490. doi: 10.1038/srep34490. PubMed PMID: 27698488; PubMed Central PMCID: PMC5048291.

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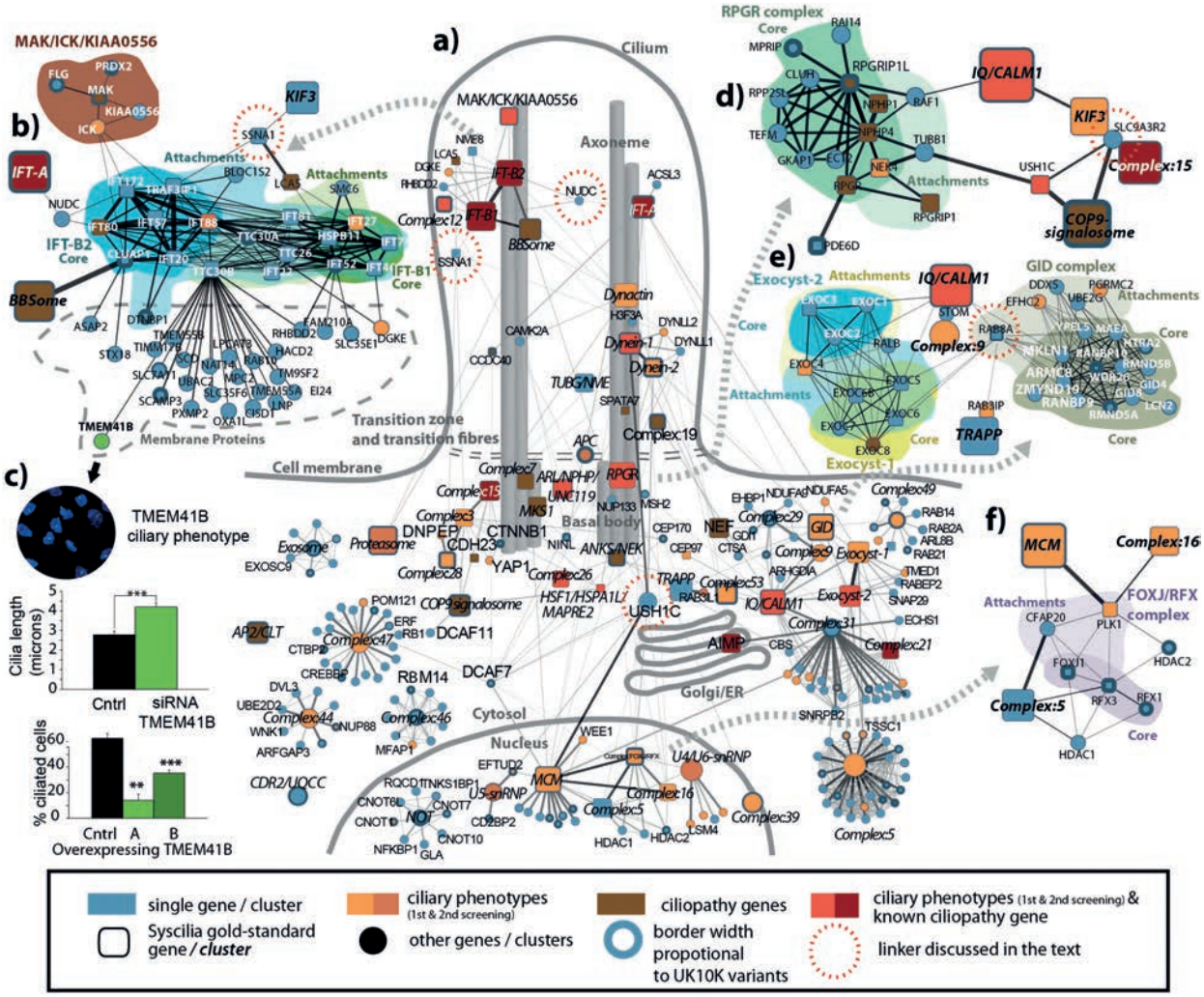

Figure 3: Overview of the ciliary landscape

a) *Complexes/proteins identified in this study. Rounded boxes show complexes (components not shown), circles denote proteins. Edge thickness is proportional to socioaffinity, and proteins/complexes are coloured according to whether they have ciliary phenotypes or variants in UK10K ciliopathy patients.* ***b,d,e,f)*** *show complexes in detail, with core and attachment subunits shaded accordingly.* ***c)*** *effect of TMEM41B siRNA knock-down or over-expression of on ciliary length.*

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